

# The Effect of Paste Concentration on Mechanical and Setting Properties of Calcium Phosphate Bone Cements

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## Abstract

Calcium phosphate cements show many advantages comparing to other calcium phosphate-based materials. The objective of this study was to investigate the influence of paste concentration controlled by liquid-to-powder ratio on compressive strength, setting characteristics and phase composition of  $\alpha$ -TCP based calcium phosphate cements. The powder phase consisted of  $\alpha$ -TCP, DCPA,  $\text{CaCO}_3$  and hydroxyapatite which were mixed with aqueous solutions of 3 wt%  $\text{H}_3\text{PO}_4$  at three different liquid-to-powder ratios (0.35, 0.40 and 0.45 mL/g). The cements were characterized in terms of setting time, compressive strength and porosity. Also XRD and SEM techniques were employed to evaluate the phase composition and surface morphology of the cements. The results revealed similar phase composition for all samples before and after soaking in Ringer's solution. Reduction in liquid-to-powder ratio led to increment of compressive strength of CPCs from about 15 MPa for the cement with L/P ratio of 0.45 to about 30 MPa for L/R ratio of 0.35 after 7 days soaking in Ringer's solution. Also by reducing the L/P ratio from 0.45 to 0.35, initial and final setting times of the CPCs decreased 3 and 5 minutes respectively. A highly microporous structure was also obtained for cements with different liquid-to-powder ratios.

## Keywords

Calcium Phosphate Cement (s); Hydroxyapatite; Bone Substitute; Bioceramics

## Introduction

In the last two decades, the most frequently investigated synthetic bone substitutes have been the Ca-P based biomaterials and especially calcium phosphate cements (CPCs) due to their superior advantages such as similarity in bone mineral composition, bioactivity, osteoconductivity and ability to form a very strong interface with bone. Blocks, granules, , coatings on dental and medical implants and cements are examples of different forms of Calcium phosphate based biomaterials (Julien et al., 2007; Van den Vreken, Pieters, Declercq, Cornelissen,

& Verbeeck, 2010).

CPCs exhibit various benefits comparing other CaP-based materials. Being injectable and easy to shape let them to be used to fill geometrically complicated bone defects (del Real, Wolke, Vallet-Regí, & Jansen, 2002). As the name suggests, CPCs consist of one or a combination of calcium phosphate powder(s) such as tetracalcium phosphate (TTCP), Hydroxyapatite (HA), dicalcium phosphate anhydrous (DCPA), dicalcium phosphate dihydrous (DCPD or brushite),  $\alpha$ -tricalcium phosphate ( $\alpha$ -TCP) and  $\beta$ -tricalcium phosphate ( $\beta$ -TCP). The powder phase upon mixing with a liquid phase like water or an aqueous solution forms a paste which based on the hardening reaction can form HA (apatite cements) or DCPD (brushite cements) after being applied within the body (Ginebra, Traykova, & Planell, 2006; Yang, Troczynski, & Liu, 2002), (Kokubo, 2008).

Nowadays,  $\alpha$ -TCP is the major component of the powder phase of many CPCs. Z. Kojic et al investigated the irritative property of  $\alpha$ -tricalcium phosphate to the rabbit skin and the results revealed that  $\alpha$ -TCP both in solid and paste form was neither toxic nor irritant (Kojic, Stojanovic, Popadic, Jokanovic, & Janackovic, 2009) .Non-toxicity, bioactivity both in vitro and in vivo and also being more bioreabsorbable than many other bioceramics such as  $\beta$ -TCP and HA has made  $\alpha$ -TCP an ideal bone substitute. (Carrodeguas & De Aza, 2011; Kojic et al., 2009) ,(Bohner, Gbureck, & Barralet, 2005; Wolke, 2001).

In clinical requirements for calcium phosphate bone cements, terms such as the initial setting time ( $S_i$ ), the final setting time ( $S_f$ ) and the ultimate compressive strength are important and have to be investigated.  $S_i$  indicates the time from whence the paste may not be deformed without damaging the structure of the

hardening cement and  $S_f$  indicates the time from whence the cement can be touched without scratching it. The clinical meaning is that the cement paste should be implanted before time  $S_i$  and that the wound can be closed after time  $S_f$  (Khairoun, 1998). On the other hand, poor mechanical properties of CPCs has limited their applications to low loaded or unloaded bearing organs (Navarro, Michiardi, Castaño, & Planell, 2008). This problem is even enhanced by the fact that the *in vivo* resorption of CPC is very slow (del Real et al., 2002). The CPCs should have compressive strength of at least about 30 MPa. The importance of other mechanical properties such as flexural strength, tensile strength, hardness and elastic modulus depends on the application environment (Wolke, 2001). So here the importance of studying parameters effecting CPC properties such as setting time and compressive strength appears. Initial setting time, final setting time and compressive strength strongly depends on various parameters including particle size of reactants, time and concentration of liquid phase and liquid to powder ratio. (Dorozhkin, 2009; Khairoun, Boltong, Driessens, & Planell, 1998).

The proportion of liquid to solid or the liquid-to-powder (L/P) ratio is a very important parameter because it determines many properties of the cement such as setting time, bioresorbability and rheological behaviour (Dorozhkin, 2009).

The aim of this work is to investigate the effect of paste concentration, controlled by liquid-to-powder ratio, on mechanical and setting properties of  $\alpha$ -TCP based cements. The rate of conversion of reactants to nano hydroxyapatite (nHA) in acellular *in vitro* medium is also studied.

## Materials and Method

### *Synthesis of $\alpha$ -TCP and nHA*

$\alpha$ -TCP was synthesized by heating 2:1 molar ratio of DCPA and  $\text{CaCO}_3$  (both from Merck, Germany) at 1400 °C for 6 hours and subsequent quenching to room temperature in air (Khairoun, Boltong, Driessens, & Planell, 1997). Then it was milled for 45 minutes in a planetary ball mill to obtain a fine particle size. The particle size distribution of  $\alpha$ -TCP was measured by laser particle size analyzer instrument (Fritsch particle sizer analysette).

Nano-crystalline hydroxyapatite was synthesized and characterized according to method described elsewhere (Hesaraki, Moztarzadeh, & Nezafati, 2009).

## *Cement Preparation*

The cement powder had the following composition: 61%  $\alpha$ -TCP, 26% DCP, 10%  $\text{CaCO}_3$  and 3% nHA (Bohner et al., 2005). In every part of study, the powder phase was mixed with liquid until a homogenous paste obtained. To investigate the effect of L/P ratio, the powder phases with the mentioned formulation was mixed with aqueous solutions of 3 wt%  $\text{H}_3\text{PO}_4$  (from Merck, Germany) at three different liquid-to-powder ratios (0.35, 0.40 and 0.45 mL/g).

## *Characterization*

### 1) *Setting Time*

Powder phase components were mixed in a mortar for about 2 min and then mixed with liquid phase thoroughly to form a homogenous paste. The initial ( $S_i$ ) and final ( $S_f$ ) setting times of the cements were measured using the Gillmore needles according to the C266-89 ASTM standard (*Standard test method for time of setting of hydraulic-cement paste by Gillmore needles.*, 1999).

### 2) *Mechanical Strength*

To prepare specimens for measuring compressive strength (CS) of the cements, the powder phase and liquid phase were mixed to form a paste and then poured into a cylindrical Teflon mold (6mm in diameter and 12mm in height). The specimens were removed from the mold after 5 h and kept in Ringer's solution for 0, 1, 4 and 7 days. A Universal Testing Machine (STM 120, Santam Co.) was used for measuring the compressive strength of specimens at the loading rate of 1 mm/min.

### 3) *Density and Porosity*

To estimate the bulk density and total porosity of the cements, the specimens were prepared in the same way as the compressive strength test specimens. Five specimens were prepared for each cement composition. The bulk density ( $\rho_b$ ) was obtained using Eq. (1):

$$\rho_b = \frac{M_c}{V_c} = \frac{M_c}{\pi r_c^2 H_c} \quad (1)$$

where  $M_c$  is the mass of the specimen,  $V_c$  its volume,  $r_c$  its radius and  $H_c$  its height. Total porosity ( $P_{\text{total}}$ ) measurements were performed on incubated dried specimens according to the density method (Takagi & Chow, 2001).  $P_{\text{total}}$  was calculated using the expression:

$$P_{\text{total}} = 100 \left( 1 - \frac{\rho_b}{\rho_p} \right) \quad (2)$$

where  $\rho_p$  is the powder density of the CPC obtained by pycnometry technique. For pycnometry measurements, the cement was ground and passed through an 800 sieve.

#### 4) Phase Analysis

The phase composition of synthesized  $\alpha$ -TCP, n-HA and the cements before and after soaking in Ringer's solution was checked by X-ray diffraction (XRD) using automated Phillips PW3710 diffractometer with Cu- $\kappa\alpha$  radiation that operated at 40 kV and 10 mA.

#### 5) Microstructure Observation

The surface morphology of the gold-coated cements was also analyzed by scanning electron microscopy (SEM, Stereoscan S360 Cambridge Ltd.) at operating voltage of 20kV and electrical current of 10mA.

#### 6) Statistical Analysis

All experiments were performed at least three times. Data were processed by Excel Microsoft 2010, and the results were presented as mean  $\pm$  standard deviation (S.D.).

### Results and Discussion

#### Characteristics of Starting Materials

Fig. 1 shows XRD spectrum of synthesized  $\alpha$ -TCP, as the major component of starting powders. All observed diffraction peaks relate to  $\alpha$ -TCP and no impurity phases are found.

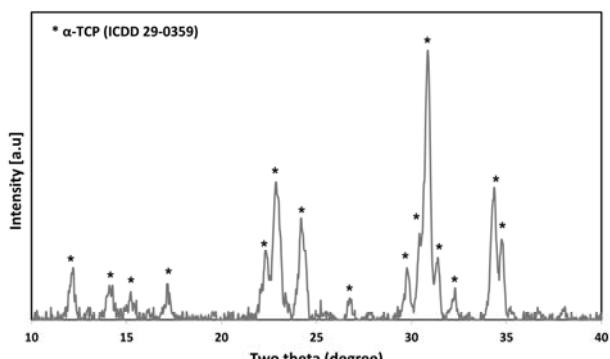


FIG. 1 XRD PATTERN OF SYNTHESIZED A-TCP

The particle-size distribution of the  $\alpha$ -TCP after being milled in planetary ball-mill for 45 minutes, obtained by laser diffraction is shown in Fig. 2. About 90% of the total volume was under 18.05  $\mu\text{m}$  diameter and mean size of the  $\alpha$ -TCP particles was about 4  $\mu\text{m}$ .

The XRD pattern of biomimetically synthesized nHA is illustrated in Fig. 3. All major peaks relate to nHA, except two peaks (at  $2\theta=29$  and 30°) which correspond

to remaining TTCP.

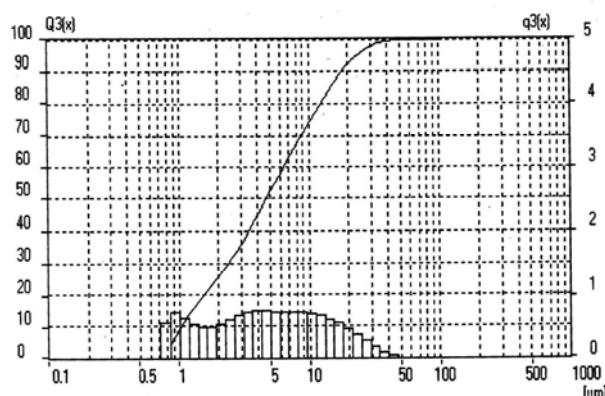


FIG. 2 PARTICLE SIZE DISTRIBUTION OF SYNTHESIZED A-TCP AFTER 45 MIN GRINDING

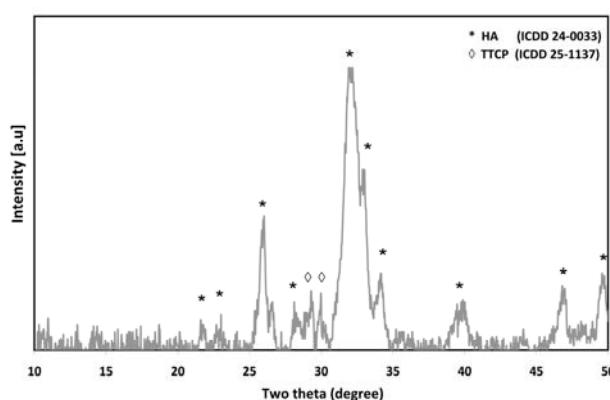


FIG. 3 XRD PATTERN OF SYNTHESIZED NANO-STRUCTURED HYDROXYAPATITE

The rod-like particles with diameter lower than 50 nm and length-to-diameter ratio higher than 3 can be observed by transmission electron microscopy as shown in Fig. 4. Some authors suggested that the HA crystals tend to grow along [0001] direction, resulting in needle-like morphology [26]. Also, nHA had a mean particle size of 1  $\mu\text{m}$ .

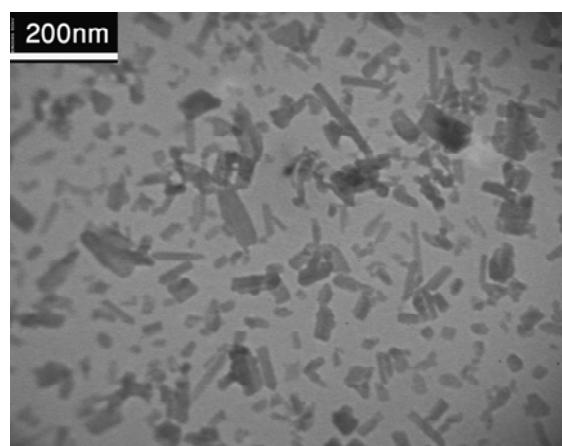


FIG. 4 TEM IMAGE FROM SYNTHESIZED NANO-STRUCTURED HYDROXYAPATITE

### Setting Time

Fig. 5 shows the initial and final setting times of the cements prepared at three different liquid-to-powder ratios. According to results, both initial and final setting times of the cements are in direct relation with L/P ratio. Although this increment in initial setting times are almost negligible, but the differences in final setting times are significant, so that the final setting time of the cements prepared at L/P ratios of 0.35, 0.40 and 0.45 mL/g are respectively 13, 15 and 21 minutes. Hence, the final setting time of the cements increased with increment of L/P ratio.

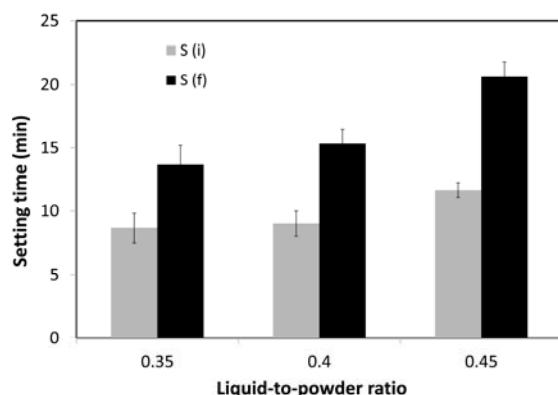


FIG. 5 INITIAL (S<sub>i</sub>) AND FINAL (S<sub>f</sub>) SETTING TIME OF CPCS PREPARED AT DIFFERENT L/P RATIOS

### Compressive Strength

Fig. 6 shows the wet compressive strength of the cements after soaking in Ringer's solution for different periods of time versus L/P ratio. The results revealed that the wet compressive strength of the cements increased with the increment of paste concentration. The wet compressive strength of specimen prepared at the L/P ratio of 0.35 mL/g was approximately twice the CPC prepared at the L/P ratio of 0.45 mL/g after 7 days of soaking in Ringer's solution.

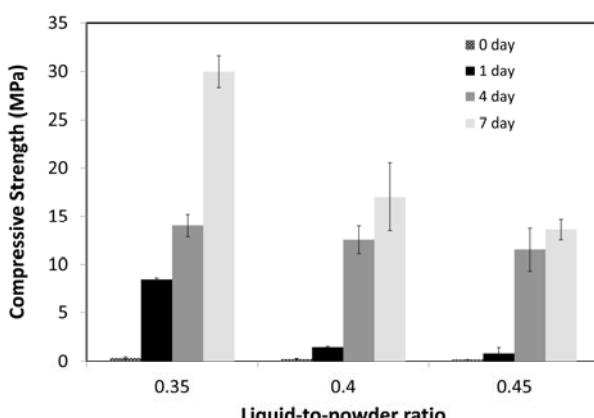


FIG. 6 COMPRESSIVE STRENGTH OF CPCS PREPARED AT DIFFERENT L/P RATIOS

Compressive strength of all cements before soaking in Ringer's solution were negligible. The compressive strength of specimens prepared at L/P ratio of 0.35 mL/g after soaking in Ringer's solution for 1 day, increased about ten times, which was much more than the increment of CS for the two other cements. The ascendant trend for CS of the specimens was continued by keeping the specimens for more days in Ringer's solution. The maximum value for CS of cements related to the specimens prepared at L/P ratio of 0.35 mL/g and soaked in Ringer's solution for 7 days (~30 MPa) which was about twice the two other cements.

### Porosity

The results related to porosity density of the cements prepared from three different paste concentration are shown in Fig. 7. Although the difference between the values was not significant, but total porosity of the cements had ascending trend by increment of L/P ratio, so that the total porosity of the cement increased from about 44% for the cement prepared at L/P ratio of 0.35 mL/g to 46% for the cement prepared at L/P ratio of 0.45 mL/g.

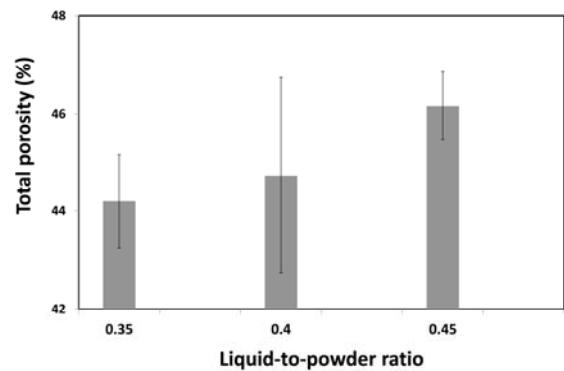


FIG. 7 INITIAL (S<sub>i</sub>) AND FINAL (S<sub>f</sub>) SETTING TIME OF CPCS PREPARED AT DIFFERENT L/P RATIOS

### Phase Composition of Cements

Comparing XRD analysis of CPCs prepared at three different L/P ratios showed that all of them had similar patterns and this means that change in L/P ratio had no effect on the final phases of CPCs. XRD pattern related to cement prepared at L/P ratio of 0.35 mL/g, soaked in Ringer's solution for 0, 1 and 7 days, is illustrated in Fig. 8. Before soaking samples in Ringer's solution (0 day) the hardened cement composed of  $\alpha$ -TCP, DCP,  $\text{CaCO}_3$  and HA which is closely similar to the cement powder. As known, the concentration of HA in cement composition is about 3 wt% and this could not be detected by XRD technique and thus the peak related to HA relates to the HA

product resulted from setting reaction. After 7 days of soaking, the diffraction peaks of hydroxyapatite with increased intensity comparing to unsoaked samples are observed (at  $2\theta=26, 32, 33, 42, 44$ ), meanwhile  $\alpha$ -TCP is still the dominant phase and conversion of starting materials to apatite has not completed yet.

### Morphology of Cements

Fig. 9 shows the surface morphology of the cements prepared with three different L/P ratios before soaking in Ringer's solution. Polyhedral particles are observed in microstructure of the cements. It seems that by decreasing the L/P ratio, the cement microstructure becomes more compacted and the size of pores decreases that is in agreement with results of porosity measurement.

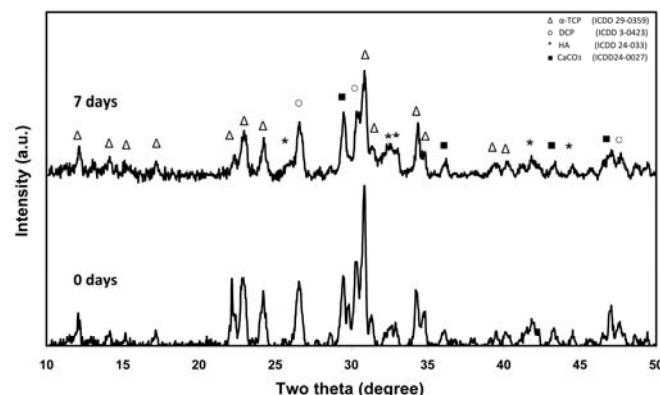


FIG. 8 XRD PATTERNS OF THE CEMENTS PREPARED AT L/P RATIO OF 0.35 ML/G AFTER 0 AND 7 DAYS OF SOAKING IN RINGER'S SOLUTION

### Discussion

Liquid-to-powder ratio of CPCs is an important parameter that influences mechanical and physical properties. Regarding to variety of CPC formulations, the role of this parameter has been investigated for TTCP-DCP based CPCs (Hesaraki, Moztarzadeh, & Sharifi, 2007)(Burguera, Xu, & Sun, 2007). In this study, the role of L/P ratio was determined on  $\alpha$ -TCP based CPCs and the results revealed that mechanical and physico-chemical properties are more influenced by changing paste concentration.

The fundamental solidifying mechanism of the calcium phosphate cements is the dissolution-reprecipitation reaction based on difference in thermodynamic stabilities of calcium phosphate salts. By mixing the powder phase of CPCs with liquid phase, the so-called dissolution-reprecipitation reaction occurs. At pH of higher than 4.2, hydroxyapatite is the most stable phase

thermodynamically. Hence, HA crystals are formed when appropriate calcium phosphate salts are mixed with the liquid phase to make the pH either neutral or alkaline.

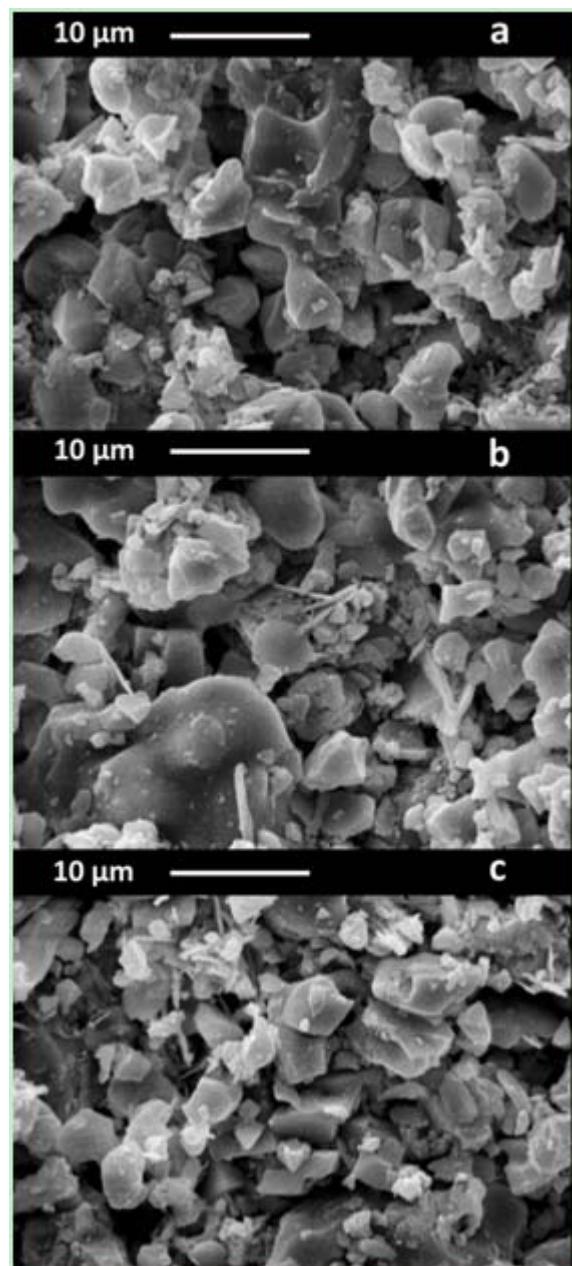


FIG. 9 SEM IMAGES OF CEMENT PREPARED AT L/P RATIO OF (A) 0.45, (B) 0.40, (C) 0.35 ML/G

The major component of powder phase in this study was  $\alpha$ -TCP which is an unstable phase in water. In other words,  $\alpha$ -TCP dissolves to supply calcium and phosphate ions. However, the solution is supersaturated with respect to the HA. Therefore, calcium and phosphate ions will be precipitated as calcium deficient hydroxyapatite (CDHA) crystals. So, the final result of the hydration reactions is a hardened mass with high strength. The use of higher amounts of

liquid in the cement (higher L/P ratios) increases the working time of the mass which delays the supersaturation of hydrate phases, which in apatite CPCs is expected to be hydroxyapatite, and this leads to longer setting times of the cements.

CPCs must set slowly enough (less than 15 minutes) to provide sufficient time to a surgeon to perform implantation but fast enough to prevent delaying the operation. Initial setting time of CPCs in this study came in range of 8 to 12 minutes.

The compressive strength of human cancellous bone ranges between 1.5 and 45 MPa. As shown in the results, the compressive strength of apatite cements normally ranges between 1 and 30 MPa, although higher values may be obtained by soaking for longer periods.

The strength of the hardened cement paste primarily depends on to its internal structure, which is determined by the shape and size of the hydration products and their arrangement and packing density (porosity). Generally, mechanical strength of CPCs is governed by two important factors: porosity and content of hydrate hydroxyapatite. Porosity has negative effect on mechanical strength whereas grown precipitated phase has positive effects. For the as-set cements, no precipitated HA can be found (as observed in XRD and SEM images) while a well-porous microstructure is observed. Thus initial compressive strength of CPCs is negligible. As reported by authors, the compressive strength of many CPCs immediately after setting is generally low. It is expected that the implanted CPC maintain its structural stability within the first implanted time while the diffusion of new tissue increases mechanical strength. When the samples are soaked in Ringer's solution, the pores provide solution permeability, precipitation of HA phases and interlocking of its crystallites that results in increment of mechanical strengths. The longer time of soaking in Ringer's solution, the more precipitated HA crystals and hence better mechanical strength of the cements.

There are more parameters other than L/P ratio that influence the strength and setting properties of CPSs, such as the cement composition, type and concentration of liquid phase, the particle size of the reactants, the crystallinity and amount of seed, and the use of liquid accelerators which in turn can be separately investigated.

## Conclusion

It can be concluded that reduction of liquid-to-powder ratio leads to increment of compressive strength of the  $\alpha$ -TCP calcium phosphate cements and also decreases both their initial and final setting time. Moreover, change in paste concentration has no effect on the final phase composition of CPCs. More compacted cement microstructure with smaller size of pores can be obtained by decreasing the liquid-to-powder ratio.

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